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SODIUM TRANSPORT IN THE FRESHWATER ASIATIC CLAM *CORBICULA FLUMINEA*

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Freshwater bivalves have a low blood-solute concentration that reduces the concentration gradients between their body fluids and the external medium and minimizes passive ion movements (Dietz and Branton, 1975; Potts, 1954; Prosser, 1973). Ion loss is still a problem due to the hyperosmotic blood and must be balanced by active ion uptake. Active Na and Cl transport systems have been reported in freshwater bivalves (Dietz, 1978, 1979; Krogh, 1939), the possible sites of Na transport being the mantle, kidney, and gill epithelia (Saintsing and Towle, 1978).

Members of the molluscan superfamily Sphaerioidea have a higher rate of Na transport than do members of the superfamily Unionoidea. The sphaeriid species *Corbicula fluminea* (= *C. manilensis*) and *Musculium transversum* (= *Sphaerium transversum*) transport Na at rates up to twice those of the Unionoidea, approaching the rates of brackish water species (Dietz, 1979). *Corbicula fluminea* is considered to be a freshwater animal although it has been reported to inhabit brackish water up to 5‰ salinity (Filice, 1958; Hayashi, 1956). Geologically, the Corbiculidae are recent invaders of freshwater (Keen and Casey, 1969).

Few physiological studies have been reported for *C. fluminea* (Dietz, 1979; Gainey, 1978a, b; Gainey and Greenberg, 1977). Because this species has been reported in both estuarine and freshwater habitats, the present study was undertaken to elucidate its mechanism of Na regulation. Sodium balance in *C. fluminea* was examined by partitioning Na flux into four processes: 1) active transport, in which ions are moved against electrochemical gradients, 2) passive diffusion, 3) exchange diffusion, an obligatory exchange of an ion with an identical ion located on the opposite side of a membrane, and 4) excretion.

Materials and Methods

Specimens of *Corbicula fluminea* were collected from local bayous and maintained unfed in an aerated aquarium containing artificial pondwater (0.5 mM NaCl, 0.4 mM $CaCl_2$, 0.2 mM $NaHCO_3$, 0.05 mM KCl). The clams were placed in a 12L:12D photoperiod at room temperature (20°–25°C) and were allowed to acclimate to laboratory conditions for at least 7 days before use. Animals undergoing salt depletion were placed in deionized water that was changed frequently. Salt depletion for more than 3 months resulted in less than 5% mortality.

Measurement of sodium flux

Sodium influx was determined from calculations based on the slope of a line representing a decrease in ^{22}Na radioactivity of the bathing medium as a function of time (Dietz, 1978, 1979; Dietz and Branton, 1975).

All experiments were begun at approximately 11 a.m. to minimize the influence of physiological rhythms (McCorkle *et al.*, 1979). Clams were placed in a de-

ionized water bath for 1 hr prior to each study to remove adsorbed ions and waste products from the valves and mantle cavity. Clams were then moved to individual 50-ml beakers containing 30–40 ml of a bathing solution. The volume of the bath depended upon the type of experiment. Except where noted, the bathing solution was Na_2SO_4 to eliminate any Na/Cl transport interactions, as the SO_4 ion is non-permeating (Dietz, 1978; Ehrenfeld, 1974). Within an hour the valves opened and the animals were siphoning the bath. Turbulence at the air-bath interface verified that the animals were in contact with and circulating the bathing medium. An initial bath sample was taken at 0 hr with a second sample taken 1–3 hr after the onset of the flux study. Sample volumes were not replaced. The time interval between samples allowed a 10–20% decrease in bath radioactivity. Bath volume and time interval were varied to prevent more than a 25% decrease in the radioactivity of the bathing medium. After the second sample was taken, the animals were removed from the bath, drained of mantle-cavity water, blotted dry, and weighed for determination of total wet weight. The valves were opened and the soft tissues removed and oven-dried at 95°C for 24 hr for measurement of dry tissue weight, shell excluded.

Sodium concentrations were measured using a flame photometer. A Triton X-100, toluene, p-terphenyl scintillation "cocktail" was added to an aliquot of each bath sample, and radioactivity was assayed by a liquid scintillation counter.

Net Na flux (J_n^{Na}) was calculated from changes in the Na concentration of the bathing medium. The net flux is positive when there is a net absorption of the ion by the animal, and negative when there is a net loss of the ion to the external medium. Unidirectional Na influx (J_i^{Na}), ions taken up from the bath by the animal, was calculated from the rate of ^{22}Na disappearance from the bath. Sodium efflux (J_o^{Na}), ions lost by the animal to the bath, was determined from the relationship $J_o = J_i - J_n$. All flux rates are given as μM Na/(g dry tissue ·hr).

Blood ion composition

Blood samples were obtained from the clams by cardiac puncture (Fyhn and Costlow, 1975). The blood was centrifuged for 2 min at 8000 × *g* to remove the cells, and the supernatant used for ion analyses. Chloride concentrations were measured by electrometric titration. Total solutes were determined from undiluted blood using a freezing-point depression osmometer.

Identification of exchange ions

The pH of the bathing medium was measured at 0 and 3 hr during Na flux study to estimate the net H efflux (J_n^H). The bath was buffered initially to pH 7.3 with 1 mM tris (hydroxymethyl) aminomethane to stabilize the pH. Bath samples were sonicated for 2 min to remove dissolved respiratory CO_2. A pH decrease was noted in each experiment. The 3-hr sample was titrated back to the original pH at 0 hr with 4.8 mN NaOH to determine J_n^H.

The release of NH_4 by the clams was assayed using the phenolhypochlorite method of ammonia determination (Solorzano, 1969). Five-ml bath samples were taken at 0 and 3 hr during an Na flux experiment. Due to tris interference in NH_4 determinations, an unbuffered 0.5 mM Na_2SO_4 medium was used. Net NH_4 flux ($J_n^{NH_4}$) was calculated from the difference between the 3- and 0-hr NH_4 concentrations.

Amiloride (0.5 and 1.0 mM/l) and ouabain (0.5 mM/l), both potential inhibitors of Na transport, were tested in the Na_2SO_4 bath as aids to identification of the possible exchange ion(s) for Na. Amiloride is thought to act on the outside surface of the epithelium, reversibly blocking the entry of Na (Ussing *et al.*, 1974). Inhibition of Na influx coupled with inhibition of H efflux has been demonstrated in crayfish, rainbow trout, frogs, and the unionid *Carunculina texasensis* (Ehrenfeld, 1974; Kirschner *et al.*, 1973; Dietz, 1978). Ouabain specifically inhibits the activity of Na-K ATPases involved in Na transport. Inhibition of NH_4 efflux by ouabain occurred in the estuarine clam, *Rangia cuneata* (Mangum *et al.*, 1978), possibly indicating an inhibitory effect of ouabain on Na/NH_4 exchange.

Partitioning of the sodium flux

Active Na transport can be measured with a radioactive tracer. To obtain a complete picture of ion balance it is necessary to quantify passive ion movements by diffusion, excretion, and exchange diffusion. Not all Na movement is active transport.

The influx (J_i) and efflux (J_o) values recorded in Tables II and III and in Figures 1 and 2 are composites of active transport, diffusion, exchange diffusion, and excretion (for efflux only), representing the total unidirectional movement of Na by *C. fluminea*. Separate studies were performed to partition the individual components of Na movement. The components of total influx (J_i^{Total}) and total efflux (J_o^{Total}) are:

J_i^{Total} = Exchange Diffusion + Passive Inward Diffusion + Active Transport
J_o^{Total} = Exchange Diffusion + Passive Outward Diffusion + Excretion.

The J_o^{Total} was calculated from the relationship: $J_o = J_i - J_n$. The diffusive and excretory Na losses cannot be separated (Dietz and Branton, 1979) and were measured together as the net loss of Na (J_n^{Na}) to deionized water. The animals were placed in individual beakers containing deionized water for 3 hr and the appearance of Na in the bath was measured. The animals were returned to pondwater for 2 days and then used in a unidirectional flux study in 0.5 mM Na_2SO_4. After an additional 2 days in pondwater J_n^{Na} was again measured in deionized water. The average value for J_n^{Na} in deionized water was defined as the combined diffusive/excretory Na component of J_o^{Total}. Since there was initially no Na in the bath to be actively exchanged for internal H or NH_4 or to participate in exchange diffusion, any Na in the deionized water at the end of 3 hr was assumed to have been lost from the clams by passive outward diffusion and/or excretion. The diffusive/excretory Na loss in Na_2SO_4 is assumed to be unchanged from the loss in deionized water. By subtracting the diffusive/excretory Na loss from J_o^{Total}, an estimated value for exchange diffusion was obtained.

The J_i^{Total} was calculated from changes in bath radioactivity and changes in the Na concentration of the bathing medium over time. By definition, the exchange diffusion out of the animal is equal to exchange diffusion into the animal. The exchange diffusion value obtained for Na efflux was inserted into the equation for J_i^{Total} as the amount of Na influx due to exchange diffusion. Transepithelial potentials (TEP) were measured *in vivo* (see below) in order to calculate the passive inward diffusion component of J_i^{Total}. Maximum passive inward diffusion of Na was calculated from Ussing's flux ratio equation (Ussing, 1949):

$$J_i^{Diffusion} = (J_o^{Diffusion+Excretion} \cdot C_o/C_i) \exp FE/RT$$

where J_o is assumed to be entirely diffusion, and C_o and C_i are the Na concentrations of the bathing medium and blood of *C. fluminea* (Table I), respectively. Faraday's constant is represented by F, E is the TEP, R is the gas constant in electrical units, and T is absolute temperature. The value for active transport was estimated by subtracting the exchange diffusion and passive inward diffusion components from J_i^{Total}.

Transepithelial potentials (TEP)

To measure the TEP, a small hole was drilled in the umbonal region of the dorsal valve approximately 4 mm ventral to the hinge line. A 2 cm piece of glass tubing was epoxied over the hole. Polyethylene tubes filled with 20 mM KCl-3% agar were connected to calomel electrodes which were, in turn, connected to a recording potentiometer. One KCl bridge was inserted through the glass tube into the body fluids. A second bridge was placed in the 0.5 mM Na_2SO_4 bathing medium as a reference. Potentials were measured when the valves were open and the clams were siphoning the bathing medium.

Statistical analyses

Student's *t* test was used to compare means of different groups of clams. The significance of the correlation between J_i^{Na} and the sum of J_n^H and $J_n^{NH_4}$ was tested using linear regression. Test statistics beyond the 95% region of acceptance were considered significant ($P < 0.05$) and statistics falling outside of the 99% region of acceptance were considered highly significant ($P < 0.01$). All data appear as mean ± 1 standard error.

Results

Effects of pondwater acclimation vs. salt depletion

A comparison of the dry tissue weights and percent body water of salt-depleted clams and clams acclimated to pondwater showed significant differences between the two groups (Table I). There was no significant difference in dry shell weights, but there was a significant difference in total wet weights. The total weight of salt-depleted clams was significantly higher than the total weight of clams acclimated to pondwater. Salt-depleted animals had a smaller dry tissue weight, but the percent body water was significantly higher than that of animals acclimated to pondwater.

Table I

Comparison of tissue weights, percent body water and blood ion composition of salt-depleted and pondwater-acclimated Corbicula fluminea. *Data are presented as mean ± SEM* (* $P < 0.05$, ** $P < 0.01$).

	Total wet weight (g)	Dry shell (g)	Dry tissue (mg)	% body water	Total blood solutes (mOsm)	mM/l	
						Na	Cl
Pond-water	5.074 ± 0.072	2.954 ± 0.049 (N = 64)	98 ± 3	95.4 ± 0.1	54.6 ± 0.6	23.5 ± 0.3 (N = 10)	24.2 ± 0.7
Salt-depleted	5.236 ± 0.113*	3.049 ± 0.070 (N = 45)	77 ± 2**	96.4 ± 0.1**	42.1 ± 0.5**	19.0 ± 0.2** (N = 10)	17.3 ± 0.4**

Table II

Sodium flux of salt-depleted and pondwater-acclimated Corbicula fluminea *in a 0.5 mM Na_2SO_4 bathing medium. Data appear as mean ± SEM (** $P < 0.01$).*

Treatment	N	μM Na/(g dry tissue·hr)		
		J_n	J_i	J_o
Pondwater	32	−2.67 ± 0.86	7.90 ± 0.79	10.57 ± 0.95
Salt-depleted	35	9.79 ± 1.23**	18.53 ± 2.10**	8.74 ± 1.27

Salt depletion caused a marked reduction in Na and Cl ions in the blood of *C. fluminea* (Table I). The total blood-solute level was reduced by 23% in salt-depleted clams, and blood Na and Cl concentrations dropped significantly. The combined decrease of Na and Cl in the blood accounted for 91% of the decrease in total blood solutes of salt-depleted clams.

Sodium flux

Salt depletion enhanced the rate of unidirectional Na uptake from an Na_2SO_4 bathing medium (Table II). The J_i^{Na} increased 234% in salt-depleted clams compared to pondwater-acclimated animals ($P < 0.01$). The J_o^{Na} was not significantly different in pondwater and salt-depleted clams. Due to increased J_i^{Na} in the salt-depleted animals, J_n^{Na} was positive.

Kinetics of sodium transport

The rate of Na influx was dependent on the external Na concentration in pondwater-acclimated and salt-depleted *C. fluminea*. A non-linear relationship was found between J_i^{Na} and external Na concentration (Fig. 1). The transport system was saturable. The maximum rate of J_i^{Na}, $\dot{V}_{max}$, for pondwater clams was 12.90 ± 3.01 μM Na/(g dry tissue·hr) in a 0.87 mM Na/l bathing solution. The affinity of the transport system, K_m, is the external Na concentration at which half of the maximum rate of Na influx occurs. The affinity of pondwater clams was 0.05 mM Na/l. Sodium influx of salt-depleted clams was variable, but the influx rates were always higher than the rates of pondwater animals in

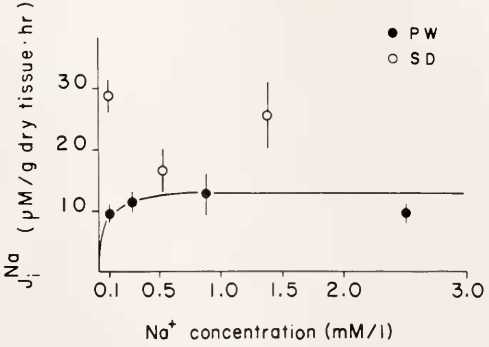

Figure 1. Effect of Na_2SO_4 concentration of the bathing medium on unidirectional sodium influx in *Corbicula fluminea*. Each point represents mean ± SEM for five animals either acclimated to pondwater (PW) or salt-depleted (SD).

TABLE III

The effects of amiloride and ouabain on sodium transport in pondwater-acclimated specimens of Corbicula fluminea. *Amiloride and ouabain were dissolved in 0.5 mM Na_2SO_4. Data are presented as mean ± SEM (* $P < 0.05$).*

Treatment	N	μM Na/(g dry tissue·hr)		
		J_n	J_i	J_o
Control	16	−0.04 ± 1.49	7.26 ± 1.01	7.29 ± 1.40
Amiloride, 0.5 mM and 1.0 mM	11	4.71 ± 1.66*	9.26 ± 1.83	4.55 ± 1.23
Ouabain, 0.5 mM	11	−3.28 ± 2.62	7.09 ± 1.13	10.37 ± 2.49

all bath concentrations tested. The maximum transport rate for salt-depleted animals was 28.66 ± 2.17 μM Na/(g dry tissue·hr). From the V_{max}, K_m was estimated for salt-depleted clams as 0.04 mM Na/l.

Effects of amiloride and ouabain on sodium transport

Amiloride at concentrations of 0.5 and 1.0 mM/l resulted in no significant changes in J_i^{Na} or J_o^{Na} and the data were pooled (Table III). Although there appeared to be a significant difference between control and amiloride-treated net fluxes, the difference may be an artifact. The fluxes from individual experiments using amiloride were not consistent. The treatment appeared to be stimulatory, inhibitory, or showed no effect on rates of Na flux at both concentrations. Ouabain (0.5 mM/l) added to the bathing solution had no effect on Na fluxes.

Partitioning of the sodium flux

The outward movement of Na to deionized water ($J_o^{Diffusion} + J_o^{Excretion}$) was 2.87 ± 0.76 μM Na/(g dry tissue·hr) for pondwater-acclimated clams (Table IV). Rates of diffusive and excretory Na loss measured in Na_2SO_4 were assumed to be unchanged from the rates measured in deionized water. The J_o^{Total} for pondwater clams was 8.94 ± 0.76 μM Na/(g dry tissue·hr). Therefore, 5.91 ± 0.80 μM Na/(g dry tissue·hr) was the estimated value for exchange diffusion. The outward diffusive/excretory component of Na movement decreased significantly ($P < 0.05$) from 2.87 ± 0.76 μM Na/(g dry tissue·hr) to 0.87 ± 0.38 μM Na/(g dry tissue·hr) in salt-depleted animals.

Total inward movement of Na, J_i^{Total}, was 8.82±0.60 μM Na/(g dry tissue·hr) for pondwater clams (Table V). The maximum passive diffusive influx was estimated with Ussing's flux ratio equation and was 0.50 μM Na/(g dry tissue·hr) for pondwater-acclimated clams (Table V). The inward diffusion of Na doubled in salt-depleted clams. The measured potential difference across the epithelia was −7.0 ± 1.2 mV (N = 10), blood negative to the bathing medium. There was no significant difference between the TEPs of pondwater-acclimated and salt-depleted specimens of *C. fluminea* and the data were pooled. The active transport component of Na movement was estimated to be 2.41 μM Na/(g dry tissue·hr) for pondwater-acclimated clams and increased fivefold in salt-depleted animals. Salt-depleted clams exhibited highly significant increases in rates of exchange diffusion, total Na influx, and total Na efflux (Tables IV and V).

Table IV

A partitioning of unidirectional sodium efflux in Corbicula fluminea. *Total sodium efflux is the unidirectional efflux measured in a 0.5 mM Na_2SO_4 bath. The diffusive/excretory component was measured in a deionized water bath. Exchange diffusion was calculated as $J_o^{Total} - (J_o^{Diffusion} + J_o^{Excretion})$. Data appear as mean ± SEM (* P < 0.05, ** P < 0.01). † Means were calculated from individual animals and this accounts for the lack of agreement with the average values.*

Treatment	μM Na/(g dry tissue·hr)		
	$J_o^{Total\ Na}$ =	$J_{oDiffusion}^{Exchange}$ +	$(J_o^{Diffusion} + J_o^{Exeretion})$
Pondwater	8.94 ± 0.76 (N = 6)	5.91 ± 0.80† (N = 6)	2.87 ± 0.76 (N = 11)
Salt-depleted	16.92 ± 0.84** (N = 7)	16.05 ± 0.67** (N = 7)	0.87 ± 0.38* (N = 14)

Exchange ions

Hydrogen and ammonium ions may be exchanged for Na in *C. fluminea*. Net ammonium flux was measured in a 0.5 mM Na_2SO_4 bath and a deionized water bath to determine the NH_4 loss through active transport and the diffusive/excretory NH_4 loss. The value of $J_n^{NH_4}$ in deionized water was not significantly different from the $J_n^{NH_4}$ in Na_2SO_4, indicating that NH_4 efflux in pondwater-acclimated clams was mostly passive and not involved in exchange for Na ions (Table VI). The $J_n^{NH_4}$ of salt-depleted animals in deionized water, 1.28 ± 0.18 μM NH_4/(g dry tissue·hr), was not significantly different from the $J_n^{NH_4}$ of pondwater animals. However, the $J_n^{NH_4}$ when salt-depleted animals were in a Na_2SO_4 bathing medium increased to 4.15 ± 0.18 μM NH_4/(g dry tissue·hr), a highly significant rise in NH_4 efflux.

Net hydrogen ion flux, J_n^H, in pondwater animals was 5.12 ± 0.53 μM H/(g dry tissue·hr) (Table VI). The J_n^H of salt-depleted animals, 7.62 ± 2.00 μM H/(g dry tissue·hr), was not significantly different from the J_n^H of pondwater animals. The sum of J_n^H and $J_n^{NH_4}$ in Na_2SO_4, 11.77 μM/(g dry tissue·hr), balanced 83% of the Na actively transported into the salt-depleted animals (Fig. 2). A linear regression of J_i^{Na} and $J_n^{H+NH_4}$ for pondwater and salt-

Table V

A partitioning of the unidirectional influx of sodium in Corbicula fluminea. *Total sodium influx is the unidirectional influx measured in a 0.5 mM Na_2SO_4 bath. Inward diffusion of sodium was calculated from the flux ratio equation. Active transport was calculated as $J_i^{Total} - (J_{i\ Diffusion}^{Exchange} + J_i^{Diffusion})$. Data appear as mean ± SEM (** P < 0.01).*

Treatment	μM Na/(g dry tissue·hr)			
	$J_i^{Total\ Na}$ =	$J_{iDiffusion}^{Exchange}$ +	$J_i^{Diffusion}$ +	$J_{iTransport}^{Active}$
Pondwater	8.82 ± 0.60 (N = 6)	5.91 ± 0.80 (N = 6)	0.50	2.41
Salt-depleted	31.42 ± 1.39** (N = 7)	16.05 ± 0.67** (N = 7)	1.17	14.20

TABLE VI

A comparison of the net NH_4 flux in 0.5 mM Na_2SO_4 and deionized water baths and net H flux in 0.5 mM Na_2SO_4 of pondwater-acclimated and salt-depleted Corbicula fluminea. *Data appear as mean ± SEM (** $P < 0.01$). Means that are not significantly different are indicated by N.S.*

Treatment	μM/(g dry tissue·hr)		
	$J_n^{NH_4}$ in Na_2SO_4	$J_n^{NH_4}$ in deionized H_2O	J_n^{H} in Na_2SO_4
Pondwater	1.17 ± 0.22 (N = 6)	1.77 ± 0.18 (N = 14)	5.12 ± 0.53 (N = 10)
	N.S.		
Salt-depleted	4.15 ± 0.18** (N = 7)	1.28 ± 0.18 (N = 14)	7.62 ± 2.00 (N = 7)
	**		

depleted animals resulted in the significant regression coefficient value $r = 0.66$ ($P < 0.02$).

DISCUSSION

Corbicula fluminea exhibits an Na transport mechanism different from that of the unionid mussels. The high J_i^{Na} and J_o^{Na} for *C. fluminea* agree with those pre-

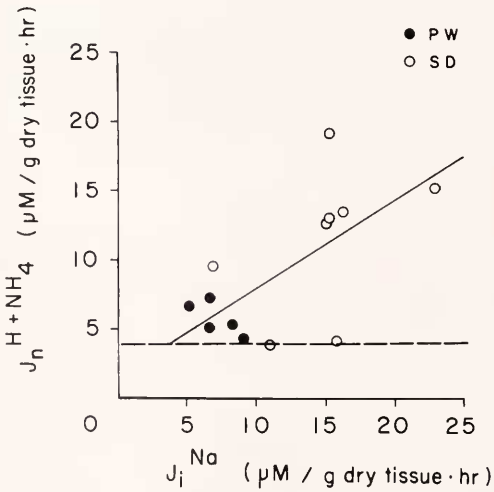

FIGURE 2. Relationship between unidirectional sodium influx and the sum of the net loss of H and NH_4 of pondwater-acclimated (PW) and salt-depleted (SD) *Corbicula fluminea* in 0.5 mM Na_2SO_4. The broken line represents the baseline excretory H + NH_4 efflux for pondwater-acclimated clams. The equation for the regression line is $J_n^{H+NH_4} = (1.48 \pm 0.80) + (0.65 \pm 0.60)J_i^{Na}$. The regression coefficient, r, is equal to 0.66 ($P < 0.02$).

viously reported by Dietz (1979). Sodium influx of *C. fluminea* is approximately six times the rate of the unionids *Carunculina texasensis* and *Ligumia subrostrata* and twice that of *Margaritifera hembeli* (Dietz, 1978, 1979). Sodium efflux is an order of magnitude higher than that of any of the unionid mussels studied by Dietz (1979). *Corbicula fluminea* also has a higher Na uptake rate relative to other freshwater invertebrates examined: 1.28 μM Na/(g wet tissue·hr) as compared to 0.29 μM Na/(g wet tissue·hr) in the gastropod *Limnaea stagnalis*, 0.65 μM Na/(g wet tissue·hr) in the crayfish *Astacus pallipes* and 0.70 μM Na/(g wet tissue·hr) (at 18°C) for the earthworm *Lumbricus terrestris* (Greenaway, 1970; Shaw, 1959; Dietz and Alvarado, 1970).

The major difference between the fluxes of *C. fluminea* and other freshwater animals is the presence of a substantial exchange diffusion component. Sixty-seven percent of the total inward Na movement is due to exchange diffusion. Only 22% of the J_i^{Na} in the crayfish and minimal amounts of the J_i^{Na} in *Carunculina texasensis* are attributable to exchange diffusion (Shaw, 1959; Dietz, 1978). In addition to the large percentage of exchange diffusion, the rate of active transport of Na into pondwater-acclimated specimens of *C. fluminea*, 2.41 μM Na/(g dry tissue·hr), is almost double the approximate value of 1.3 μM Na/(g dry tissue·hr) recorded for the unionids (Dietz, 1979). Absence of the active transport component could place the animals in a negative ion balance, depending on the rate of Na efflux relative to the inward Na movement.

The magnitude of active Na transport depends upon the degree of salt depletion. Table II indicates transport rates for animals salt-depleted for an average of 40 days. Although the fluxes were not partitioned for these studies, the active transport component is at least equal to the net Na uptake. The clams used for the partitioning studies were salt-depleted for a longer period (about 90 days) and display a further elevation of active Na transport (Table V). For all studies, Na-Na exchange diffusion is about half of the total Na influx. Exchange diffusion is characteristic of corbiculid Na transport.

Non-equilibrium conditions may be inferred by comparing observed TEP with TEP estimated for animals in a steady-state condition. In a steady state Ussing's flux ratio equation simplifies to the Nernst equation:

$$E = (RT/F) \ln (C_i/C_o),$$

giving the TEP (E) necessary to maintain Na in electrochemical equilibrium. If there is no active transport, observed TEP would equal the TEP estimated by the Nernst equation. The estimated TEP of −74 mV does not equal the observed TEP of −7 mV, suggesting active Na transport in *C. fluminea*. Previous studies on other freshwater clams have indicated that TEP is a calcium-diffusion potential independent of Na, Cl, or SO_4 (Dietz and Branton, 1975).

Transport of Na in *C. fluminea* is efficient. The affinity of the transport system for Na ions in these animals is greater than the affinity in the unionid mussels. Even with twice the active transport rate the affinity, K_m, in *C. fluminea* is 0.05 mM Na/l as compared to the K_m of 0.15 mM Na/l in *Carunculina texasensis* (Dietz, 1978). The K_m in *C. fluminea* is low relative to literature K_m values of 0.2–0.7 mM Na/l in other freshwater animals (Dietz and Alvarado, 1970, 1974; Greenaway, 1970; Maetz, 1973; Prosser, 1973; Shaw, 1959). Sodium influx increases two to three times in salt-depleted animals without a significant change in K_m, suggesting that more epithelial transport sites are activated during the salt-depletion treatment. Passive diffusion and/or excretion of Na out of the salt-depleted animal is reduced (Table IV).

The active transport systems in *Carunculina texasensis* and several other freshwater animals are primarily Na/H exchanges with minimal involvement of NH_4 as an exchange ion (Dietz, 1978, 1979; Ehrenfeld, 1974; Kerstetter *et al.*, 1970; Maetz, 1973; Maetz *et al.*, 1976). *Corbicula fluminea* acclimated to pondwater does not differ from other freshwater animals in this respect. The $J_n^{NH_4}$ of pondwater-acclimated clams appears to be primarily excretory since no change in NH_4 output was noted between Na_2SO_4 and deionized-water bathing solutions. Although the J_n^H was not measured in a deionized water bath, the excretory J_o^H may be estimated. Assuming that Na/H exchange occurs as a 1:1 ratio, active Na transport may be subtracted from total J_n^H, leaving 2.71 μM H/(g dry tissue·hr) as the excretory output. Na/H exchange has been reported to occur on a 1:1 basis in two species of amphibia and in crayfish (Garcia Romeu *et al.*, 1969; Garcia Romeu and Ehrenfeld, 1975; Ehrenfeld, 1974). The baseline excretory H + NH_4 efflux for pondwater animals may then be approximated as 4.12 μM/(g dry tissue·hr) (Fig. 2).

Salt-depleted and pondwater-acclimated specimens of *C. fluminea* show essentially the same $J_n^{NH_4}$ in deionized water; however, the $J_n^{NH_4}$ for salt-depleted clams in Na_2SO_4 quadrupled. Although Na/H exchange appears to be the primary exchange mechanism under normal conditions (Maetz and Garcia Romeu, 1964; Maetz *et al.*, 1976), *C. fluminea* may be activating a Na/NH_4 exchange as an auxiliary mechanism under stress of salt depletion. The active transport exchange ratio of Na: (H + NH_4) was 2.2:1 in salt-depleted clams. The lack of stoichiometry suggests there may be a change in epithelial Na permeability when animals are in Na_2SO_4 solutions. The calculated exchange diffusion in Na_2SO_4 may therefore by underestimated and active Na transport overestimated.

Salt depletion is often used to stimulate active Na uptake in freshwater animals, but important changes in the animals should be recognized. Salt depletion caused a significant reduction in dry tissue weight of *C. fluminea*. Concurrently, the percent body water increased. The total blood-solute level was reduced, mostly by highly significant reductions in Na and Cl concentrations. These animals have undergone salt-depletion in the laboratory for long periods of time without mortality, but part of the elevated Na influx may be due to the 20% loss in dry tissue weight. The small tissue weight of this particular clam species makes the changes in weight and body water even more important. Significant loss of dry tissue weight is correlated with elevated NH_4 efflux in salt-depleted clams. Tissue protein hydrolysis generates free amino acids, which may become major intracellular solutes contributing to water gain. Free amino acids are probably a major energy source under these laboratory conditions. Catabolism of amino acids would generate the additional H and NH_4 necessary for Na exchange in the salt-depleted clams.

Specimens of *Corbicula fluminea* show little sensitivity to amiloride and ouabain as inhibitors of Na transport. The apparent lack of inhibition in pondwater-acclimated animals may be a result of the small fraction of total Na turnover attributable to active Na transport. These data suggest a fundamental difference in the mechanism of Na–Na exchange diffusion and active Na transport, rather than exchange diffusion being an artifact of active transport.

The auxiliary Na/NH_4 exchange in salt-depleted animals may have been an important exchange mechanism when *C. fluminea* inhabited brackish water. Ammonia efflux against a gradient has been reported in the marine invertebrates *Rangia cuneata*, *Nereis succinea*, and *Callinectes sapidus* (Mangum *et al.*, 1976,

1978). The large exchange-diffusion component of *C. fluminea* might also be a vestige of its brackish water habitation. Exchange diffusion in estuarine blue crabs living in freshwater is reported to comprise 50% of Na influx (Cameron, 1978). Exchange diffusion in the unionids, long term inhabitants of freshwater (Haas, 1969), is negligible. Many of the differences in the Na balance mechanism of *Corbicula fluminea* as compared to other freshwater animals may be attributable to its recent brackish water heritage.

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Summary

The Na transport mechanism was examined in pondwater-acclimated (PW) and salt-depleted (SD) specimens of *Corbicula fluminea*. The Na influx in 0.5 mM Na_2SO_4 of 7.90 ± 0.79 μM Na/(g dry tissue·hr), higher than most freshwater animals, increased to 18.53 ± 2.10 μM Na/(g dry tissue·hr) in SD animals.

Saturation of the transport system is typical of Michaelis-Menten enzyme kinetics. Maximum influx of PW clams was 12.90 ± 3.01 μM Na/(g dry tissue·hr), with a K_m of 0.05 mM Na/l. The maximum rate in SD clams was 28.66 ± 2.17 μM Na/(g dry tissue·hr), with little change in K_m.

Sodium movement in *C. fluminea* may be partitioned into passive diffusion, excretion, exchange diffusion and active transport. Exchange diffusion comprises a substantial portion of Na movement: 5.91 ± 0.80 μM Na/(g dry tissue·hr) in PW animals and 16.05 ± 0.67 μM Na/(g dry tissue·hr) in SD clams. Passive inward diffusion of Na was 0.50 μM Na/(g dry tissue·hr) for PW clams and 1.17 μM Na/(g dry tissue·hr) for SD clams.

The primary exchange ion for Na is H, although a Na/NH_4 exchange is functional in SD animals. In PW clams, 2.41 μM H/(g dry tissue·hr) is transported in a 1:1 exchange with Na. In SD clams, the net NH_4 flux quadrupled contributing to a Na: ($H + NH_4$) exchange.

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